Differences between female and male patients with familial rheumatoid arthritis

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Abstract
Objective—To determine whether there are genetic differences between female and male patients with familial rheumatoid arthritis (RA).
Methods—45 men and 119 women from 78 families with RA who all had at least one first degree relative with RA were compared. HLA-DRB1 alleles were analysed, including DRB1*04 subtypes and associations of DRB1*04 haplotypes with DQB1*0301 or DQB1*0302 alleles, the age of the patients at disease onset, the presence of rheumatoid factor (RF), joint erosions, and rheumatoid nodules.
Results—HLA-DRB1*13 allele (the subtype allele of DR6, reported to be protective against the development of RA) was found in 14/119 (12%) of female but in none of the male patients (p=0.036). The HLA-DR4 allele was found slightly more often in men than women patients with familial RA (31/45 (69%) v 75/119 (63%), NS). Men were also more often RF positive than women (44/45 (98%) v 98/117 (84%); p=0.031). On the other hand, the mean age at onset of RA was significantly lower in the female group (40.4 years) than in men (46.6 years, p=0.0044).
Conclusion—The results indicate that there is stronger genetic background in familial male than female patients with RA in the genetic susceptibility defined by the studied HLA antigens. However, the earlier age of onset of the disease in female group and the increased proportion of women with RA indicate that there are additional sex related predisposing factors enhanced in familial cases.

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It has been suggested that families with an excess of affected men having a young age at onset may be the most informative in identifying the genetic factors either predisposing to or protecting from the development of RA.² However, it is difficult to find a representative, sufficiently large number of families with an excess of affected men to carry out such studies. On the other hand, it seems that in some family studies the age at onset of RA was often younger in women than in men, though not always reaching statistical significance.^{8–10} In the family study by Khan *et al* no difference in the age at onset of RA between familial male and female patients with RA was found.⁷

One might expect that genetic factors accumulate in families with several members affected by RA. We could also show this for HLA-DR risk and protective alleles in our multicase family series, which we have now analysed for possible sex related differences. We compared the frequencies of HLA-DRB1 antigens, including HLA-DRB1*04 subtypes and DRB1*04 associated DQB1 alleles, ages at the onset of disease, and the presence of rheumatoid factor, bone erosions, or rheumatoid nodules in men and women.

Patients and methods

PATIENTS

We collected data on 78 Finnish families, in which at least two family members (first degree relatives to each other) had RA. Data of the index cases were collected from six different rheumatological units (Turku, Paimio, Satalinna, Tampere, Jyväskylä, and Oulu). Patients with RA who had at least one first degree relative with the disease were asked by questionnaire to participate in the study. The 78 families comprised 57 affected sib pairs, 16 parent-child pairs, and five sib trios. Additionally, there were also two children of two index cases affected and one mother of another index case affected in another sib pair. Rheumatoid factor (RF) status was not known for two female patients and in one male and one female patient the existence of rheumatoid nodules was unknown. Knowledge of erosions was missing in one female patient. Altogether 45 men and 119 women patients with familial RA, including index cases and affected family members, were included in this study.

All patients fulfilled the American College of Rheumatology (formerly the American Rheumatism Association) criteria for classification of RA.¹¹ Clinical documentation of all study patients included sex, age at disease onset (that is, at the time of diagnosis of RA), disease

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Table 1 Mean (SD) age of patients at disease onset (in years) in familial male and female RA groups

	Males [n]	Females [n]	p
All	46.6 (13.1) [45]	40.4 (14.3) [119]	0.0044
RF* positive patients	46.7 (13.2) [44]	39.2 (13.1) [98]	0.0019
RF negative patients	45.0 [1]	46.4 (18.9) [19]	NS
DR4 positive	47.2 (13.1) [31]	39.7 (14.9) [75]	0.0057
DR4 negative	45.5 (13.5) [14]	41.6 (13.3) [44]	NS

*RF = rheumatoid factor.

duration, serum RF status, and the presence of joint erosions or rheumatoid nodules. The patients were classified as seropositive if there had been one positive RF test at any time during the disease course. The test results for RF positivity and radiographs for erosions and existence of rheumatoid nodules were re-evaluated from the case records.

HLA TYPING

DNA was extracted from anticoagulated blood samples by a salting out method. The HLA-DR alleles were determined by a sequence-specific polymerase chain reaction (PCR) amplification. The low resolution genomic typing used could identify HLA antigens from DR1 to DR18 and also DR52 and DR53. When a single HLA-DRB1 allele could be amplified, the alleles were assumed to be homozygous. The presence of HLA-DQB1 alleles *0301 or *0302, or both, was defined by

a method based on hybridisation of PCR products with a panel of lanthanide labelled oligonucleotide probes, and the HLA-DR4 subtypes were defined by a similar method designed for this purpose.¹⁰

STATISTICAL METHODS

Statistical analyses were performed with the χ^2 test with continuity correction when differences in proportions of HLA frequencies, sex, RF, rheumatoid nodule, or bone erosion status between the familial female and male patients were compared. Differences in the age at onset of disease and in the disease duration were analysed by a Mann-Whitney U test.

ETHICAL ISSUES

The study was accepted by the ethical committees of participating hospitals, and the samples were collected after informed consent.

Results

The male patients with familial RA were more often RF positive than female patients with familial RA. Only one seronegative man among 45 cases (2%) was found compared with 19/117 (16%) women ($p=0.031$). Seronegative women were less often DR4 positive than RF positive women. Thus, in particular, the number of seronegative HLA-DR4 negative women was higher, nine out of 117 (8%) than the corresponding number in men (1/45 (2%), NS).

The HLA-DR4 antigen was slightly more common in familial men than women (31/45 (69%) *v* 75/119 (63%)), but the difference did not reach statistical significance. The difference in the frequency of the so-called shared epitope (SE) common to DRB1*0401/*0404/*0405/*0408/DR1 and DR10 alleles, which was found in 40 (89%) male and 99 (83%) female patients, also did not reach significance. On the other hand, the HLA-DR13(6) antigen was found in 14/119 (12%) women but in none of the male patients ($p=0.036$). The frequencies of all other studied HLA-DR alleles were comparable in male and female patients with familial RA. The same HLA comparisons were also made by analysing only probands from each family. Similar trends were found, though the differences were not statistically significant owing to the smaller numbers.

The HLA-DRB1*0401 allele was the most common DR4 subtype in both groups, and no significant difference in the distribution of the subtypes was found between male and female patients with familial RA. We also determined the presence of HLA-DRB1*04-DQB1*0301 and DRB1*04-DQB1*0302 haplotypes in HLA-DR4 positive patients. Its presence was comparable in men and women (data not shown).

In men and women there were no differences in the proportions of patients with erosions (36/45 (80%) and 102/118 (86%), respectively) or rheumatoid nodules (7/44 (16%) and 21/118 (18%), respectively). Nevertheless the mean age at onset of RA was lower in the female group (table 1) and therefore the disease duration in them was also longer than

Figure 1 Age at disease onset in (A) male and (B) female patients with familial rheumatoid arthritis.

in the male group (12.1 years *v* 17.3 years, $p=0.092$). The age difference at disease onset was statistically significant also when only subjects positive for RF and HLA-DR4 were compared. The distribution of the ages at disease onset is also shown in fig 1, which clearly demonstrates the earlier onset of disease in the female group. Our patients included 35 patients from different generations (16 parent-child pairs, two affected children of probands, and one affected mother of a proband). To exclude the possible bias caused by these patients we analysed the results for the mean age of different patient groups at disease onset also including only patients in the same generation. In that comparison there were 38 male and 91 female patients with RA. All the results were essentially similar to those in the whole patient group ($p=0.012$ for all patients in the same generation and $p=0.0061$ for those positive for RF and HLA-DR4).

Discussion

This study searched for evidence of possible genetic heterogeneity between male and female patients with familial RA. Interestingly, although it seems that familial male patients have more HLA associated genetic background, we found that the mean age of patients at disease onset was significantly lower in the female group. This suggests the presence of additional genetic predisposing factor(s) in the familial female group. We think that those additional genetic factor(s) are common in the female patients with RA and, as suggested by Jaraquemada *et al* in 1986, explain the finding that more women than men develop RA despite the fact that both groups show a significant increase of DR4 frequency.³ The lower age at onset in the familial female group may indicate that the additional genetic predisposing factors are enhanced in familial cases at the same time as other predisposing factors (common to both sexes) are accumulating. In our previous study we did not find a lower age of onset in the non-familial female RA group.¹⁰

It has been suggested earlier that HLA genes are more important for the development of RA in men than in women.³ The decreased frequency of HLA-DRB1*13 and slightly increased frequency of the HLA-DRB1*04 in our male patients with familial RA as compared with the female patients is in accordance with this hypothesis. The numbers are still small and the *p* value for the difference in DRB1*13 is significant only when calculated without correction for the number of comparisons made. However, we detected a protective effect of HLA-DRB1*13 in RA in both familial and sporadic cases,¹⁰ and this has also been found in several earlier studies, though not always reaching a statistical significance.^{12–14} The effect

might also be due to the associated DQ alleles, which have been suggested to be of primary importance in genetic protection against the disease.¹⁵

This study showed an increased frequency of RF positivity in the male patients compared with the female patients; the same phenomenon has been reported earlier in patients with sporadic RA.⁴ The reason for this higher incidence of RF in male patients with RA is not known but may reflect the effect of the difference in the genetic background of the sexes in the pathogenesis of RA. There may exist a seronegative non-HLA associated disease, more often affecting women, which would have profound differences in its aetiopathogenesis.

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